TRANSACTIONS
OF
American Microscopical Society
(Published in Quarterly Instalments)

Vol. XXXIX APRIL, 1920 No. 2

MODERN DARK-FIELD MICROSCOPY AND THE HISTORY OF ITS DEVELOPMENT

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INTRODUCTION

In most work with the microscope the entire field of view is lighted and the objects to be studied appear as colored pictures or as shadows—in extreme cases, as silouhettes—on a white ground. As the field is always light, this has come to be known as Bright-Field Microscopy (Fig. 1).

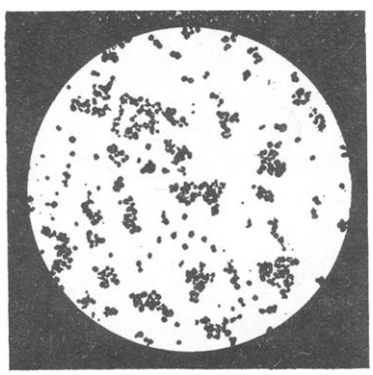

Fig. 1

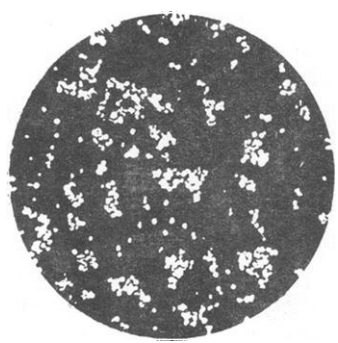

Fig. 2

Bright- and dark-field photo-micrographs of the same objects (starch grains).

In contrast with this is Dark-Field Microscopy in which the field is dark, and the objects appear as if they themselves emitted the light by which they are seen (Fig. 2).

The study of objects in a bright-field probably comprises 95% of all microscopic work, and is almost universally applicable. On the other hand dark-field microscopy has only limited applicability, and yet from the increased visibility given to many objects it is coming to be appreciated more and more.

Definition.—In its comprehensive sense, Dark-Field Microscopy is the study of objects by the light which the objects themselves turn into the microscope, and none of the light from any outside source passes directly into the microscope as with bright-field microscopy.

There are two principal cases: (A) The objects which are truly self-luminous like phosphorescent animals and plants; burning or incandescent objects, and fluorescent objects. (B) The objects which emit no light themselves, but which deflect the light reaching them from some outside source into the microscope.

These two groups are well represented in Astronomy. If one looks into the sky on a cloudless night, the fixed stars show by the light which they themselves emit, but the moon and the planets appear by the light from the sun which they reflect to the earth, the sun itself being wholly invisible at the time. As there is relatively very little light coming from the intervening space between the stars and planets, all appear to be self-luminous objects in a dark field. This reference to the sky at night will serve to bring out two other points with great clearness: (1) The enhanced visibility. Everybody knows that there are as many stars in the sky in the daytime as at night, but they are blotted out, so to speak, by the flood of direct light from the sun in the daytime, while at night when these direct rays are absent and no light comes from the back-ground the stars and the planets show again by the relatively feeble light which they send to the earth.

(2) The other point is that in dark-field microscopy the objects must be scattered, not covering the whole field (Fig. 2). If there were no intervening empty space the whole face of the sky would look bright

It will be seen from this that ordinary sections or other objects so large that they fill the whole field of the microscope cannot be studied advantageously by the dark-field method, for they would make the whole field bright. But for the liquids of the body, blood, lymph, synovial, and serous fluids, fluid from the cavities of the

nervous system, saliva, and all other mucous fluids, and isolated tissue elements where the solid or semi-solid substances are distributed in a liquid, the appearances given by this method are a revelation as was pointed out by Wenham and Edmunds and many others over fifty years ago. No less is the revelation coming from the study of bacteria, protozoa and other micro-organisms in the dark field.

Dark-Field and Ultra-Microscopy

In both of these the objects seem to be self-luminous in a dark field, and no light reaches the eye directly from an outside source, but only as sent to the eye from the objects under observation.

The terms simply represent two steps, and merge into each other.

Dark-Field Microscopy deals with relatively large objects, 0.2μ or more in diameter, that is, those which come within the resolving power of the microscope.

Ultra-Microscopy deals with objects so small that they do not show as objects with details, but one infers their presence by the points of light which they turn into the microscope. This can be made clear by an easily tried-naked-eye observation. Suppose one is in a dark room, and a minute beam of brilliant light like sunlight or arc light is directed into the room. Unless one is in the path of this beam of light it will remain invisible, but if there are vapor or dust particles present they will deflect some of the light toward the eye and will appear as shining points. The character of the particles cannot be made out, but the points of light they reflect indicate their presence. As Tyndall used this method in determining whether a room was free from dust in his experiments in spontaneous generation, the appearance of the shining dust particles is sometimes called the "Tyndall effect."

The two forms are said to merge, because in studying objects like saliva, etc., with the microscope designed especially for dark-field work, some of the objects seen will show details, but some are so small that they show simple as points of light usually in the form of so-called diffraction discs. The larger objects in the saliva come in the province of dark-field microscopy, and the smallest ones, of ultra-microscopy, and in this case the instrument used might with equal propriety be called a dark-field or an ultra-microscope.

The great purpose of the dark-field microscope is to render minute objects or details of large objects plainer or actually visible

from the advantages offered by the contrast given between the brightly lighted objects and the dark background. For example, with the homogeneous immersion objective the study of fresh blood with the ordinary bright-field method enables one to see the red corpuscles with satisfaction, but the leucocytes are not easily found and the blood-dust (chylomicrons) and the fibrin filaments are not seen at all or very faintly. With the same microscope using the dark-field illumination the leucocytes are truly white cells, and the blood-dust is one of the striking features of the preparation, and the fibrin filaments seem like a delicate cobweb.

In this connection, perhaps a few words should be added on the terms Resolution and Visibility. Both came over from the ancient science of astronomy, and are properly used only when restricted as in astronomy.

By resolution is meant the seeing of two things as two, not blended. For example if two stars are close together they are resolved if they appear as two. When the telescope was invented it was found that many stars that appeared single were really two stars close together. If two lines are placed close together they appear as two to the naked eye when close up, but as one moves away the lines seem to fuse and make one. Visibility refers only to the possibility of seeing a thing. In the above examples the twin stars were visible to the naked eye but not resolved into two, and likewise the lines were long visible after they could be seen as two lines. Now the purpose of the ultra-microscope is solely to increase the visibility of small particles without reference to their details of structure. Dark-field microscopy, on the other hand, while it gives greatly increased visibility, also gives resolution of details.

As with bright-field microscopy the resolution of details of structure depends directly upon the numerical aperture (NA) of the objective, and the brightness upon the square of the aperture (NA^2).

Method of Dark-Field Microscopy

In this article the ultramicroscope and the study of self-luminous objects will not be further considered, but the discussion will be limited to objects which must be lighted by some outside source.

There are two principal cases: (1) objects which are lighted from above the stage of the microscope or by so-called direct light (Fig. 3)

and, (2) objects which are lighted from below the stage, or by transmitted light (Fig. 4).

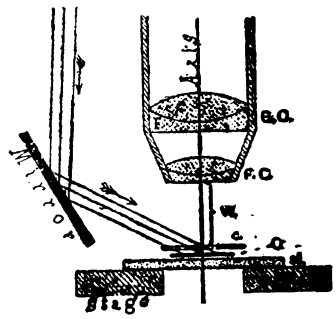

Fig. 3. Light from above the stage. (From *The Microscope*)

In both cases the light from the source is at such an angle that none of it can enter the objective directly but only as it is deflected or "radiated" by the objects in the microscopic field.

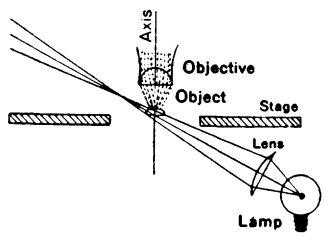

Fig. 4. Light from below the stage.

When the light upon the object is from above the stage the background must be non-reflecting. If the background were white there would be a kind of bright-field, not dark-field microscopy.

The black-background is secured either by placing the object directly upon some black velvet or other non-reflecting surface, or on a glass slide which in turn is placed upon black velvet, etc., or on a dark well. The simplest way to produce a dark-well is to turn the condenser aside and place a piece of black velvet over the foot of the microscope. Or the condenser can be lowered well and the velvet put over the top of the condenser.

Diffuse daylight from a window, or more satisfactorily, artificial light directed by a mirror or lens (bull's eye), is directed obliquely down upon the preparation (Fig. 3). Exactly the same preparation will answer for light from below the stage. In this case the condenser is turned out of the way, and some black-velvet put over the foot of the microscope to cut out stray light.

For a good naked eye demonstration showing the increased visibility due to the dark-field, some cotton may be placed on a piece of black velvet, and a similar tuft of cotton on a white card.

For the special methods of lighting microscopic objects from above the stage, see in the historical summary at the end of this paper.

Dark-Field Microscopy by Transmitted Light.—To make objects appear self-luminous in a dark field when illuminated by beams of light from below the stage, two things are necessary:

(1) The objects must be able to deflect in some way the light impinging upon them into the microscope.

(2) None of the light from the source must be allowed to pass directly into the microscope. These conditions are met when (a) the objects to be studied are of different refractive index from the medium in which they are mounted, and (b) when the transmitted light thrown upon the object is at such an angle that it falls wholly outside the aperture of the objective (Fig. 4-7).

The objects deflect the light into the microscope

(1) By Reflection
(2) By Refraction
(3) By Diffraction

Any one of these will suffice, but any two or all of the ways may be combined in any given case.

For low powers where the aperture of the microscope objective is relatively small it is comparatively easy to make the transmitted beam of so great an angle that none of it can pass directly into the microscope. A simple experiment will show this: A 16 mm. or lower objective is used, the substage condenser is turned aside and on the stage is placed a clean slide with a little starch, flour, or other white powder dusted upon it. If now the mirror is turned to throw the light directly up into the microscope the field will be bright and the objects relatively dark, but if the mirror is turned at an angle suf-

ficient to throw the whole beam at a greater angle than the aperture of the objective will receive, the field will become dark and the starch or flour grains will stand out as if shining by their own light. If some black velvet is placed on the foot of the microscope so no light can be reflected upward into the microscope from the foot or the table, the field will be darker. This experiment succeeds by either natural or artificial light. If some water containing paramecium and other micro-organisms is put on the slide and put under the microscope, the organisms will appear bright and seem to be swimming in black ink.

It is readily seen that with the method just discussed the light is all from one side (Fig. 4). To light the objects from all sides, that is, with a ring of light, the simplest method, and the method utilized in all modern dark-field microscopy, is to use a hollow cone of light, the rays in the shell of light all being at so great an angle with the optic axis of the objective that none of them can enter the microscope directly (Fig. 4-7).

With Refracting Condensers. With the condensers of the achromatic or chromatic type used for bright-field microscopy a solid cone of rays is used. To get the dark-field effect the objects to be studied must be lighted only by the rays at so great an angle that they cannot enter the objective directly. This requires that the condenser shall have a considerably greater aperture than the objective. The ordinary method of making the hollow cone is to insert a dark stop—central stop—to block or shut off the central part of the solid cone of light. The object is then illuminated with a ring of light of an aperture greater than that of the objective (Fig. 6). Some of this light is turned by the objects into the microscope. As only a relatively small amount of the light is deflected by the objects into the microscope, it is evident that there must be a great deal of light to start with or there will not be enough passing from the object to the microscope to make it properly visible. The question also naturally arises how one is to determine the size of the central stop to be used with any given condenser and objective.

This is easily determined as follows: The field is lighted well as for ordinary bright-field observation and some object is got in focus. Then the object is removed and the iris diaphragm of the condenser opened to the fullest extent. If one then removes the ocular and looks down the tube of the microscope and slowly closes

the iris, when the full aperture of the objective is reached, that is, when the back lens of the objective is just filled with light, the opening in the iris represents the size of the central stop to use to cut out all the light which would pass into the microscope from the condenser; all the ring of light outside of this is of too great an angle for the aperture of the objective. One can measure the size of the opening in the iris with dividers and then prepare a central stop diaphragm.

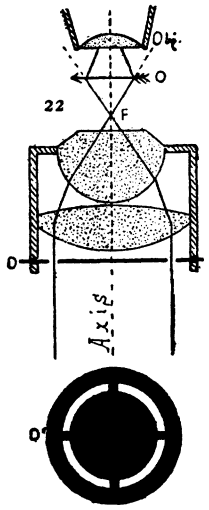

Fig. 5. Ordinary condenser with sectional and face views of the central stop (D). (From *The Microscope*)

A visiting card is good for this. It should be blackened with India ink. To be on the safe side it is wise to make the central stop a little greater in diameter than the iris opening (Fig. 5).

If now the microscope is lighted as brilliantly as possible, and then the iris opened to its full extent and the blackened central stop is put in the ring under the condenser, and a slide used with starch or flour on it, the flour or starch particles will be lighted with the ring of light, and they will deflect enough into the objective to make the objects appear bright as if shining by their own light, the background remaining dark. If the field looks gray or light instead of black it is because the central stop is too small or not centered or the particles used for objects are too numerous, not leaving enough blank space.

One can determine what is at fault thus: The ocular is removed. If the central stop is too small the back lens of the objective will show a ring of light around the outside. If the central stop is not centered there will be a meniscus of light on one side. If the objects are too numerous the whole field will be bright. To verify these statements one can use a specimen with flour or starch all over the slide. It will look dazzlingly light, with the ocular in place and the back-lens will be very bright when the ocular is removed.

For the meniscus of light when the central stop is decentered, purposely pull the ring holding the stop slightly to one side and the meniscus will appear in the back lens. To show the ring of light due to a too small size of the stop, the easiest way is to use a higher objective, say one of 3 or 4 mm. in place of the 16 mm. objective. While it is necessary to eliminate all the light which could enter the objective directly, the thicker the ring of light which remains to illuminate the objects the more brilliantly self-luminous will they appear, therefore one uses only the stop necessary for a given objective. If one makes central stops for the different objectives as described above it will be greatly emphasized that the objectives differ in aperture, in general the higher the power the greater the aperture, and consequently the larger must be the central stop, and the thinner the ring of light left to illuminate the object. As one needs more light for high powers instead of less than for low powers, the deficiency of light caused by the large central stop must be made good by using a more brilliant source of light for the high powers.

Reflecting Condensers. As was first pointed out by Wenham, 1850-1856, refracting condensers are not so well adapted for obtaining the best ring of light for dark-field work as a reflecting condenser, on account of the difficulty in getting rid of the spherical and chromatic aberration in the refracted bundles of such great aperture. He first (1850) used a silvered paraboloid and later (1856) one of solid glass as is now used. Within the last 10-15 years there has also been worked out reflecting condensers on the cardioid principle. The purpose of all forms is to give a ring of light which shall be of great aperture, and be as free as possible from chromatic and spherical aberration, and hence will form a sharp focus of the hollow cone upon the level where the objects are situated.

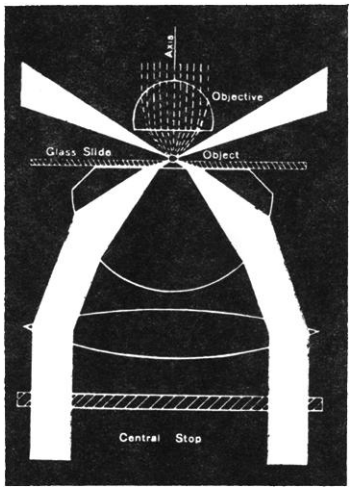

Fig. 6. Bright-field condenser with central stop to give dark-field illumination.

This is a sectional view showing the hollow cone of light focusing on the object and then continuing wholly outside the aperture of the objective.

The light deflected by the object into the objective is represented by broken lines.

The glass slide is in homogeneous contact with the top of the condenser, and the medium beyond the object is represented as homogeneous with glass.

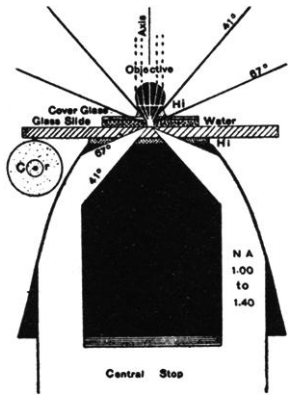

Fig. 7. Paraboloid condenser for dark-field illumination.

Axis—The principal optic axis of the microscope.

Central Stop—The opaque stop to cut out all light that would be at an aperture less than 1.00 NA.

Cover Glass—The cover for the object. For dry objectives it must conform to the objective, and with homogeneous objectives it must be less than their working distance in thickness.

C r—Face view of the top of the paraboloid showing the centering ring, the spot of white ink in the middle and the grains of starch for centering and focusing high powers.

Glass Slide—The slip of glass on which the object is mounted. It is connected with the top of the paraboloid by homogeneous liquid, and must be of a thickness to permit the focusing of the hollow cone of light upon the object.

Hi, Hi—Homogeneous liquid between the cover-glass and the objective and between the top of the condenser and the slide.

NA 1.00 to 1.40—The numerical aperture of the hollow cone of light focused on the object by the paraboloid. As indicated on the left this is represented by a glass angle of 41 to 67 degrees.

41° 67°—The limits of the angle of the rays in glass. Objective—The front lens of the objective. The light rays deflected by the object are indicated by white lines below and through the lens, then by broken, black lines above the front lens of the objective. Water—The mounting medium for the objects.

In this diagram the course of the rays from the paraboloid are indicated as if the objects were mounted in homogeneous liquid and that the rays passed beyond the focus into a medium homogeneous with glass.

TABLE SHOWING THE MAXIMUM ANGLE IN GLASS, AND THE CORRESPONDING NUMERICAL APERTURE OF THE LIGHT WHICH CAN PASS INTO MEDIA OF DIFFERENT REFRACTIVE INDEX ABOVE THE CONDENSER (FIG. 8-11)

	Angle in Glass	Numerical Aperture	Index of Refraction
1. Air over the condenser	41°	1.00	1.00
2. Water	61°	1.33	1.33
3. Glycerin	75° 15′	1.47	1.47
4. Homogeneous liquid	90°	1.52	1.52

In the reflecting as in the refracting condensers the central part of the light beam from the source is blocked out by a central stop and only a ring of light enters the condenser.

Immersion connection of condenser and glass slide bearing the specimen.—While the purpose of the reflecting condenser is to produce a very oblique beam of light for illuminating the objects, it is seen at once that the laws of refraction will prevent the light from passing from the condenser to the object unless the glass slide bearing the object is in immersion contact with the top of the condenser.

That is, for air (index 1.00) above the condenser, the rays in glass at 41°, NA 1.00 and less can pass from the condenser into the air and expand into a hemisphere of light in it (Fig. 8). Rays above 41° are totally reflected back into the condenser.

For water (index 1.33) above the condenser, rays in the glass at 61°, NA 1.33, and less can pass into the overlying water and make a complete hemisphere of light in it (Fig. 9). Rays above 61° are totally reflected back into the condenser.

For glycerin (index 1.47) above the condenser, rays in the glass at 75° 15', NA 1.47 and less can pass from the glass into the overlying glycerin and form a hemisphere of light in it (Fig. 10). All rays at a greater angle are reflected back into the condenser.

For homogeneous liquid (index 1.52) over the condenser, there is no limit to the angle of light that can pass from the condenser to it (Fig. 11).

Immersion Liquid between Condenser and Glass Slide. While water or glycerin answers fairly well it is recommended that homogeneous liquid be used in all cases. At first glance this would seem unnecessary for, as just stated the aperture of the light is limited by the medium of least refractive index between the condenser and the object. Thus objects mounted in watery fluids, and especially those mounted in air would seem to have the illuminating ray that could reach them limited by an aperture of 1.33 in one case and of 1.00 in the other (glass angles of 61° and 41°). This would be true if the objects were suspended in the water or in the air, but many of the particles are not suspended but rest on the glass slide, that is are in so-called *optical contact* with the slide. This being true, the angle of the light which can pass from the condenser to them depends upon their own refractive index, and not upon that of the mounting medium (air or water). This explains also why objects not in optical contact with the slide are rendered more visible by the homogeneous immersion contact of slide and condenser for the scattered light from the particles in optical contact helps to light up particles not in contact.

Another consideration also favors the use of the homogeneous immersion contact of slide and condenser, even for objects mounted in air. Physicists have found (see Wood) that beyond the critical angle, while all light is turned back into the denser medium, it does nevertheless pass one or more wave lengths into the rarer medium to

find, so to speak, an easier place to turn around in. If now any object is near enough the slide to fall into this turning distance of the totally reflected light it may be said to be in optical contact, and the light which meets it will pass into it instead of being totally reflected.

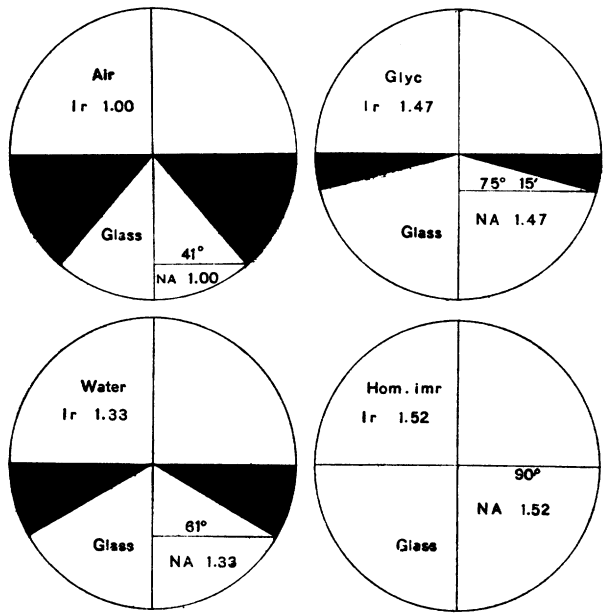

Fig. 8, 9, 10, 11. Diagrams showing the angle and numerical aperture of the light in glass to fill the entire hemisphere above, with overlying media of air, water, glycerin, or homogeneous immersion liquid.

As shown by the diagrams, the NA of the light in each case must equal the index of refraction (Ir) of the overlying medium to fill the overlying hemisphere with light. If the light is at a greater than the critical angle it is reflected back into the condenser. Such light is represented by black in 8, 9, 10. With homogeneous liquid (Hom. imr) above the condenser there is no critical angle.

It should be said in passing that the medium of least refractive index in the path of the light beam from the condenser determines the critical angle at which the light is wholly reflected, and hence determines the maximum angle of the illuminating pencil that can light the object, but this does not apply if the object is in optical contact with the glass (see below).

One can make a very convincing experiment to show the importance of remembering that some of the objects are in optical contact with the glass slide and hence may utilize light which could not pass

into the surrounding medium. If the upper face of the dark-field condenser is cleaned as perfectly as possible, and then lighted well, one can see no light emerging from the top except where the centering ring is situated or where there are some accidental scratches. If one dusts some starch, flour or other white powder on the clean surface, the particles which make optical contact with the glass will glow as if self-luminous. In case one wishes further evidence, the end of the condenser should be carefully cleaned, and a glass slide of the proper thickness connected with it by means of homogeneous liquid, then some flour or starch can be dusted on the slide and it will glow as did the particles on the top of the condenser. These demonstrations show well with the naked eye and with objectives up to 8 mm. (Fig. 7, Cr.)

Aperture of the Ring of Light in the Condenser. As the angle of the light illuminating the objects must be greater than can enter the objective employed it follows that the central part of the illuminating beam must be blocked out up to or beyond the aperture of the objective to be used. The greatest aperture rays possibly attainable depends upon the opticians ability to so design and construct the condenser that it will bring the remaining shell or ring of light to a focus. For those designed to be used with all powers, the aperture of this ring of light usually falls between 1.00 NA and 1.40 NA. As water and homogeneous immersion objectives have a numerical aperture greater than 1.00 NA. it follows that they could not be used for dark-field observation with their full aperture, because much of the light from the condenser could enter the objective, giving rise to a bright or at least a gray field.

Reducing diaphragms for high apertured objectives. As the lower limit in aperture of dark-field condensers is 1.00 NA, and sometimes even lower, it follows that a condenser for use with all objectives requires that none of them have an aperture over 1.00 NA. As all modern immersion objectives have an aperture greater than 1.00 NA, this aperture must be reduced by inserting a diaphragm in the objective.

The general law that the resolution varies directly with the aperture, and the brilliancy as the square of the aperture, holds with dark-field as with bright-field microscopy. In order to determine by actual experiment with various dark-field condensers the best aperture of the diaphragm to select, the writer requested, the Bausch & Lomb Optical Company and the Spencer Lens Company to supply

reducing diaphragms for their fluorite, homogeneous immersion objectives ranging from 0.50 NA. to 0.95 NA. As measured by me these diaphragms ranged from slightly above 0.50 NA, to 0.97 NA. These varying apertures were tested on each condenser, using the same light and as nearly as possible identical preparations (i.e., fresh blood mounted on slides of the proper thickness). It seemed to the

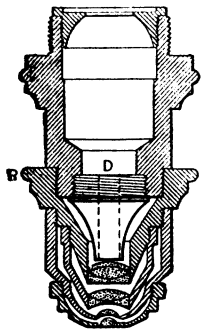

Fig. 12. Large aperture objective with diaphragm to reduce the aperture to less than 1.00 NA. (From Chamot)

D Funnel-shaped reducing diaphragm in the interior of the objective above the back lens.

writer that the law of aperture as stated above held rigidly. The question then is, which aperture shall be chosen if but one diaphragm is available? It seemed to the writer that the one of 0.80 NA should be chosen, at least for these fluorite objectives. If three are to be had the range should be 0.70, 0.80 and 0.90. The reason why one over 0.90 is not recommended is because some examples of the best of the dark-field condensers tested, seemed to have their lower limit somewhat below 1.00 NA, and hence the field could not be made completely dark with the diaphragm of 0.97 NA. With others, however, the field was as dark with this large aperture as with the lower apertured diaphragms.

A considerable range of reducing diaphragms for the homogeneous immersion objectives is recommended because all experience brings home to the worker with the microscope the conviction that some structures show better with the lower apertures and some with higher ones, and it is believed from considerable experience that the same fundamental principles hold in dark-field as in bright-field microscopy.

Lighting for Dark-Field Microscopy

As is almost self-evident, only a very small amount of the light passing through the condenser to the objects is deflected by the objects into the microscope, consequently the source of light must be of great brilliancy or there will not be enough to give sufficient light to render the minute details of the objects visible, when high powers are used. This visibility of minute details involves three things: (1) The aperture of the objectives; (2) The aperture of the illuminating pencil; (3) The intensity of the light.

The most powerful light is full sunlight. Following this is the direct current arc, the alternating current arc and then the glowing filament of the gas-filled or Mazda lamps.

The reflecting condensers are designed for parallel beams consequently the direct sunlight can be reflected into the condenser with the plane mirror of the microscope. If the arc lamp, a Mazda lamp, or any other artificial source is used a parallelizing system must be employed. The simplest and one of the most efficient is a plano-convex lens of about 60 to 80 mm. focus with the plane side next the light and the convex side toward the microscope mirror (Fig. 14) i.e., in position of least aberration. This is placed at about its principal focal distance from the source whether that be arc lamp, Mazda lamp, or any other source and the issuing beam will be of approximately parallel rays. These can then be reflected up into the dark-field condenser with the plane mirror.

Lamps for Dark-Field Microscopy

Up to the present the small arc lamp (Fig. 13), using 4 to 6 amperes is practically the only one considered really satisfactory. There is no question of the excellence of the direct current arc. The alternating current arc has two equally bright craters which renders its use somewhat more difficult.

For most of the work in biology the arc gives more light than is comfortable to the eyes; but a still greater objection is that with the burning away of the carbons the source of light is constantly shifting its position, and hence the quality of the light varies from minute to minute. A third difficulty for hand-feed lamps is that one must stop observation frequently to adjust the carbons.

In spite of all these difficulties, however, the arc lamp is indispensable if one desires to attack all the problems for which the dark-field microscope is available.

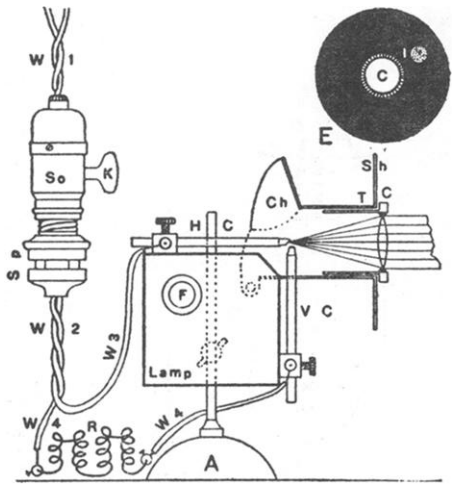

Fig. 13. Small arc lamp for dark-field illumination (From *Optic Projection*)

This figure is to show the wiring necessary and the arrangement of the arc and lens to give a parallel beam.

A—Heavy base of the lamp support. By means of a clamp the lamp can be fixed at any desired vertical height. HC and VC, the horizontal and vertical carbons. The HC must be made positive. F, the wheels by which the carbons are fed.

TC—The tube containing the condenser. The condenser in the inner tube can be moved back and forth to get a parallel beam. Sh, black shield, see E.

E—Black shield at the end of the lamp tube (Sh). It serves to screen the eyes and to show when the spot of light is thrown back by the mirror into the parallelizing lens.

W1, W2, W3, W4—The wires of the circuit passing from supply to the upper carbon (HC) and from the lower carbon (VC) to the rheostat, and from the rheostat back to the supply in W1. Never try to use an arc lamp without inserting a rheostat in the circuit. As shown, it forms a part of one wire. It makes no difference whether it is in the wire going to the upper or to the lower carbon, but it must be in one of them.

6-Volt Headlight Lamp.—Next to the arc lamp in excellence for dark-field work is the 6-volt gas-filled headlight lamp (Fig. 14). The reason of this excellence is that the filament giving the light is in a very close and small spiral not much larger than the crater of the small arc lamp, and hence approximates a point source of light.

The brilliancy is also very great as the filament is at about 2800° absolute. The two sizes that have been found most useful by the writer are the bulbs of 72 watts and those of 108 watts. For the bulb of 108 watts a mogul socket is essential; for the 72 watt bulb the ordinary socket is used.

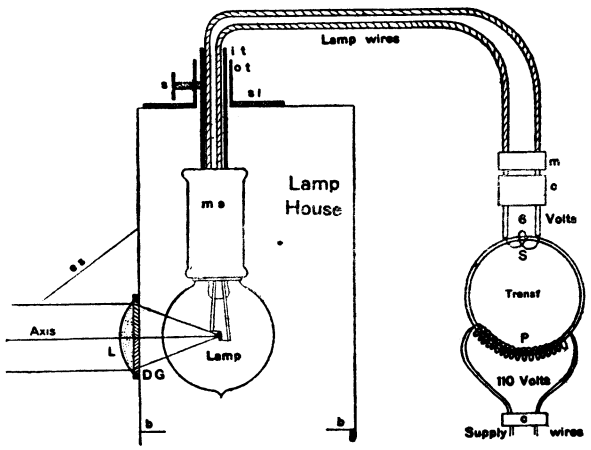

Fig. 14. Diagram of headlight lamp and transformer for dark-field illumination (About one-sixth natural size).

Axis—Axis of the parallel beam from the lens (L).

Lamp—The 6-volt, 108 watt headlight lamp with its very small, close filament centered to the axis of the lens. It is in a mogul socket (ms) and can be centered vertically and horizontally by the inner and outer tubes and set screw (it, ot, s), and the brass slide (sl).

Lamp House—The metal container for the lamp. (b b) Bafle plates near the bottom to help avoid stray light. At the left over the lens (L) is the sloping eye shade. L D G—Parallelizing lens cemented to polished daylight glass.

Lamp wires—The large wires from the transformer (Transf.) to the lamp (Double heater wires are good).

m c—Mistakeless connection between the lamp wires and the transformer (Transf). This is a Manhattan stage connector, and is different from anything else in the laboratory and therefore the lamp can never be connected with a 110 volt circuit and burn out the lamp. Of course any other wholly different connection would answer just as well.

Transf.—Diagram of a step-down transformer. As there are 18 coils around the soft iron ring on the Primary (P) or 110 volt side, and but one coil around the Secondary (S) side, the voltage is stepped down 18 times, or from 110 to 6 volts. In an actual transformer the coils would be far more numerous, but in this proportion. If the transformer were connected wrongly, i.e., with the lamp wires connected with

the primary (P) side, and the 110 volt supply with the secondary (S) side, it would then be a step-up transformer, and raise the 110 volts 18 times—with disastrous results. C, separable connection for the 110 volt supply wires.

The only difficulty with these lamps is that as they are for a 6 volt circuit it is necessary to use a step-down transformer if one has an alternating current with a voltage of 110 or of 220, as is usual.

If one has a direct current of 110 or 220 voltage, then it is necessary to use a storage battery, in general like those used for the lighting and ignition systems of automobiles. As a transformer uses up but a very small amount of energy it will be readily seen that in stepping down the voltage the amperage is correspondingly raised from the general law that the wattage is the product of the voltage into the amperage, and knowing any two the third may readily be found.

For example with the 72 watt lamp, if the voltage is 6 the amperage must be 72/6 or 12 amperes. With the 108 watt bulb the amperage must be $108/6 = 18$ amperes.

The heating of the filament is determined by the amperage, and also it must be remembered that the conductor of an electric current must be increased in due proportion for an increased amperage, consequently in the transformer the wires joining the 110 volt line is small because a very small amperage is necessary to give a large wattage; while from the transformer to the lamp the conducting wires must be large, to carry without heating the amperage necessary with the low voltage (6) to give the large wattage (108 or 72).

For the 18 amperes of the 108 watt bulb, the Fire Underwriters specifications call for wire of No. 12 or No. 14 Brown and Sharp Guage, i.e., wire 1.6 to 2 mm. in diameter or a cable composed of smaller wire having the same conductivity. This specification is for continuous service. In wiring the headlight lamp from the transformer, so called *heater cable* is good, provided one uses a double cable, that is the entire cable for each wire. This is easily done by removing the insulation at the ends and twisting the two strands together, then it can be treated as one wire and the two thus treated used to join the lamp to the mistakeless connection (m c, Fig. 14, 15) of the transformer. As the resistance is small in these large conductors the full effect of the current remains to make especially brilliant the glowing lamp filament, and brilliancy is what is needed for this work.

It should be stated that the transformer for this purpose should be substantial and adapted to continuous service. It is known as a "Bell Transformer" as it is connected to ordinary house light systems for ringing door bells. The one used by the writer was obtained from the General Electric Co. in 1920 and costs at present seven dollars. It is marked: Transformer, type N D, Form P Volts 110 6. Capacity 108 KV-A, Cycles 60, Without taps in Primary." (For making the connections, see the explanation of Fig. 14.)

In comparing the two 6 volt lamps for dark-field work, the 72 watt lamp answers well for most purposes, but the 108 watt one approximates more nearly to the small arc lamp and is sufficient for probably 99% of all dark-field observation in biology. For the remaining 1% one could safely depend on sunlight.

Stereopticon and Mazda lamps for dark-field. In absence of the head-light lamps described above, one can get good results by using in the lamp-house (Fig. 14-15), a stereopticon lamp bulb of 100 to 250 watts. These bulbs have the filament arranged in a kind of ball, and hence fairly well concentrated. This filament must be centered with the parallelizing lens as described for the headlight bulbs. For the horizontal position, move the lamp back and forth by the brass slide until the front of the ball filament is in focus on the 10-meter screen. The microscope should then be placed from 15-25 cm. from the lamp-house. The rest of the procedure is exactly as for the headlight lamp.

If one has neither headlight lamp nor stereopticon lamp, still good work can be done in biology by using the Mazda C bulbs where the filament is in the form of a loop or C. This is centered and focused as for the other lamps (Fig. 18). If one has only a lamp similar to Fig. 18, the daylight glass can be removed and the microscope placed close to the lamp. Fairly good results can be obtained with a 100 watt mazda stereopticon or c bulb without a parallelizing lens.

The Spencer Lens Company recommend in addition to the small arc lamp, their small magic lantern (No. 394). This has either a 250 or a 400 watt stereopticon lamp bulb, and for parallelizing system, the two plano-convex lenses common with simple magic lanterns. The projection objective of the magic lantern is removed. This yields good results especially when a piece of clear daylight glass

is placed over the end of the cone left vacant by the removal of the objective.

A real advantage possessed by these different lights is that the lamps are connected directly with the 110 volt circuit, no transformer being required, as with the headlight lamps. But if one is to do much dark-field work the headlight lamps are much to be preferred.

Daylight effects with the headlight or Mazda lamps. For dark-field work as for work with the bright field, daylight effects are of the greatest advantage both for eye comfort and for the clearness with which details can be made out. The daylight effect is readily obtained by using a piece of daylight glass polished on both sides and cemented to the flat face of the parallelizing lens by means of Canada balsam (Fig. 14-15).

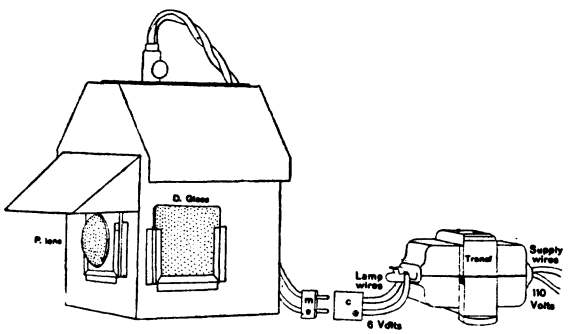

Fig. 15. Headlight lamp in its metal house, and the step-down transformer. (About one-eighth natural size)

D. Glass—The window of daylight glass on the side of the lamp-house to be used for bright-field work. With the glass removed the centering of the lamp is facilitated.

P. lens—Parallelizing lens of about 75 mm. focus. It is cemented to a piece of polished daylight glass.

m c—Mistakeless connection between the lamp wires and the transformer (Transf.). Such a connection prevents joining the lamp with the 110 volt circuit, and thus burning it out. This cannot be connected wrongly.

Transf.—Step-down transformer from 110 to 6 volts.

Lamp-House with centering arrangement. To avoid the non-utilized light, and to place the source of light in the most favorable position, there must be an opaque box to enclose and support the head-light or Mazda lamp. As the filament giving the light must be in the optic axis and practically in the focus of the parallelizing

lens, the lamp or the lens must be sufficiently movable to attain the end. In the lamp-house here figured (Fig. 14, 15) the lens is stationary and the lamp is movable horizontally and vertically, that is, it can be raised and lowered and moved toward and from the lens in the optic axis. For the most perfect centering there should also be arrangements for moving the lamp or the lens from side to side. In the one here shown the parallelizing lens can be shifted slightly to take care of the lateral centering.

Centering the Lamp-filament. As stated above the lamp-filament must be centered, that is, put in the principal optic axis of the parallelizing lens. This is most satisfactorily done by putting the parallelizing lens in position in the lamp-house and measuring the distance from the table to the middle point of the lens. The middle point of lamp filament should be placed at the same height from the table. This is easily accomplished by using the side window of the lamp-house and raising and lowering the lamp by means of the vertical adjustment (Fig. 14-15) until the filament is at the right height to be on the level of the optic axis. Then the lamp is turned until the spiral filament faces the lens. The two limbs of the fork holding the filament then face sidewise. Of course, they would make a shadow if they faced the lens.

To get a parallel beam. The most satisfactory way of doing this is to work at night or in a dark room. Having a white wall or white screen at about 10 meters distant, light the lamp and move it back and forth in the optic axis by means of the top slide (Fig. 14 sl) until the filament of the lamp is in focus on the screen, the filament will then be at about the principal focus of the parallelizing lens, that is, in a position to give approximately parallel light to the microscope. It is well to mark the position on the top of the lamp-house so that if it gets accidentally displaced it can be returned without trouble. It may be said in passing that the lamps are not all exactly alike so that when a new lamp is installed it is necessary to center and focus all over again.

Focusing the crater of the small arc lamp. The makers arrange the carbons and the lens tube so that the crater will be approximately in the optic axis (Fig. 13). Now to get the crater in the focus of the parallelizing lens one can proceed in principle as with the headlight lamp. In the arc lamp, the carbons are fixed and the lens movable. Work at night or in a dark room and with the lighted arc move the

lens back and forth until there is a sharp image of the crater on the 10-meter screen.

Lighting the Microscope. Assuming that the lamp filament or the crater of the arc lamp is centered with the parallelizing lens, one can find the best position for the microscope by holding some thick white paper in the path of the beam and slowly moving out along the beam. Where the spot of light is brightest and most uniform is the best place for the microscope mirror. With the headlight lamps and the arc light this is usually 20-30 cm. from the parallelizing lens.

To get the spot of light to fall on the 45° mirror properly, the center of the mirror must be at the level of the axis of the beam. This can be brought about either by raising the microscope on a block, by inclining the microscope, or by tipping the lamp-house over toward the microscope. If some white paper is put over the mirror one can tell easily when the cylinder of light falls upon it.

To get the light up through the condenser and into the objective it is necessary to so tip the mirror that an image of the source of light is directed back into the parallelizing lens. This image is reflected back from the flat top of the condenser to the mirror. With this arrangement of the mirror the microscope is almost always well lighted, and the mirror will need but a slight adjustment to give the best possible light. This will only be true however, when the source of light is centered to the parallelizing lens and the condenser to the axis of the microscope.

This method of lighting the microscope saves much time and worry. It is effective with the microscope vertical or inclined, with the lamp-house vertical or inclined, and finally it is unnecessary to have the microscope in line with the beam of light. It may be at right angles or at any angle provided the beam of light falls directly on the mirror and the image of the source can be reflected back to the parallelizing lens.

This method of lighting the microscope, so simple and generally applicable, has the one draw-back that the reflected image is rather faint and therefore not easily seen in a light room; at night or in a dark room it is very easily applied. If one is using the headlight lamp and the parallelizing lens is on the outside as shown in Fig. 14-15, one can tell easily when the image is reflected back into the lens from the bright image seemingly considerably nearer the

lamp filament than the blue image of the filament shown in the lens. To see these images one should look obliquely into the lens, that is, along a secondary not along the principal axis. One can also gain help in lighting by turning the mirror till a spot or ring of light appears on the upper end of the condenser. If the slide is in place with the oil for immersion, the spot of light will be bright. One must usually change the mirror slightly after the preparation is in focus to get the best light.

Centering and Focusing the Dark-Field Condenser

As can be seen by Fig. 6-7 the object must be in the focus of the dark-field condenser and this focus must be in the optic axis of the microscope.

The dark-field condenser must have a special mounting with centering screws, which is the common method; or if the microscope has a centering sub-stage arrangement the dark-field condenser need not have a special centering arrangement, but be put in the centering substage fitting. Ordinarily there is no centering arrangement on a microscope and hence the dark-field condenser must have a special centering arrangement of its own. The whole is then placed in the usual bright-field substage condenser ring and raised until it is at the level of the top of the stage. As a guide to centering, there is a circle scratched on the upper surface of the condenser (Fig. 7 c-r). With a low power (16 mm. objective or lower, and x5 ocular) one focuses down on the end of the condenser and if the small circle is not concentric with the circle of the field the centering screws are used with the two hands at the same time and adjusted until the circles are exactly even all around. Unfortunately this is not sufficient for the most satisfactory work, as it is rare that any two objectives will be exactly centered even though screwed into the same opening in the nose-piece, and much less likely to be centered if in different openings. To get the best results the objective to be used and the dark-field illuminator must be centered to each other. To accomplish this the following procedure has been found simple and certain: To start with the dark-field condenser is centered by the low objective as described above, and then with a crow-quill or other very fine pen one puts a very small point of Chinese white or other white ink in the middle of the little centering circle. This is

easily done if an objective of 20 to 40 mm. focus is used for centering the circle on the condenser.

Now for centering the oil immersion or other high power objective the field of which is less than the centering circle, the objective is put in place, but no immersion liquid need be used for the centering. The top of the condenser has dusted upon it some starch or flour or other fine white powder so that in focusing down upon the top of the condenser there will be some shining particles to focus on if the white ink in the center of the circle should happen to be entirely out of the field, which is often the case. When the objective is in focus the centering screws are used to shift the condenser until the minute spot of white ink in the center of the circle is exactly in the middle of the field. In this way any objective may be centered with the condenser, and so far as the centering is concerned, one can be sure of getting the best results of which the condenser is capable.

When the condenser is centered to the high objective, the starch particles and the white ink may be removed with a piece of moist lens paper or a soft cloth.

Focusing the Condenser on the Object Level. This is one of the most essential steps for good dark-field work. If the objects are not in the focus of the condenser they will not be sufficiently lighted so that they can radiate enough light into the microscope to show all their details.

One can proceed as follows, it being assumed that the preparation is mounted on a slide of the proper thickness for the given condenser:—Use a low power, 16 to 50 mm. objective and light the microscope as described in the preceding section. Look into the microscope and focus on a saliva preparation. Move the slide around until there are plenty of epithelial cells in the field and then make slight changes in the mirror until the most brilliant light is obtained. With the screw device for raising and lowering the condenser shift the position up and down slightly until the smallest and most brilliantly lighted point is found. When this is accomplished the condenser is in the optimum focus for that slide and will give the most brilliant light of which it is capable for the source of light used.

Any preparation for examination can have the condenser focused upon it as just described.

For experimental purposes a very satisfactory preparation for focusing the condenser is made as follows: A slide of the right thick-

ness is selected and cleaned and on one face near the middle is painted, with a fine brush, a very thin layer of Chinese white or other white ink. When this is dry, a drop of Canada balsam is put upon it and then a cover-glass. The white particles are very fine and serve admirably to show the focal point of the condenser. Such a slide can be kept as a standard and if the condenser is focused by its aid, it will be in the right position for any preparation mounted upon a slide of the same thickness as the standard. One must always remember, however, that many preparations have an appreciable thickness, and if the slide were of exactly the same thickness as the standard the light might be made more brilliant in a given case by focusing the condenser slightly upward for the higher levels of the preparation. This shows also that the slides selected for preparations should be somewhat under the maximum thickness allowable for the given condenser.

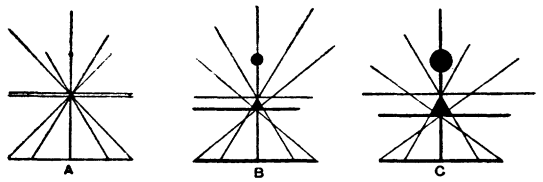

Fig. 16. Face and sectional views of the focus of the hollow cone of light from dark-field condensers

A—Sectional view of an optically perfect dark-field condenser in which the sun is represented as focused nearly to a point. No such condenser exists.

B—Sectional view of a possible condenser focus. It is drawn out somewhat and spreads laterally. The variation in the thickness of slide which might properly be used is shown by the two parallel lines enclosing the elongated focus.

C—Sectional view with a still more elongated focus. The parallel lines show that the variation in thickness of slide permissable is correspondingly increased.

The apparent size of the sun's image is shown on the axis above in each case. It is least sharp in C.

The black line above the letters (A, B, C) represents the top of the condenser.

Thickness of glass-slide to use. Mention has been made of glass slides of the proper thickness. What should this thickness be and how can it be determined are pertinent questions for one who is to get satisfactory results in dark-field work. The thickness of the slide with any given condenser is that which will bring the focus of the condenser—that is the image of the source of illumination—on the upper face of the glass slide where the object is located. Either

on the instrument or in the maker's directions for its use the thickness of slide which should be used with it is given. If such definite information is not available or if a person wishes to determine for himself the proper thickness of slide to use, it may be found out as follows: An arc lamp and a dark room are necessary. The light should preferably be parallelized as shown in Fig. 13. The tube of the microscope is removed, and a piece of uranium glass with plane faces is placed on the stage and connected with the top of the condenser by homogeneous immersion liquid. The uranium glass is strongly fluorescent and shows with great definiteness the exact path of the beams of light from the condenser. One can see exactly where the light comes to a focus above the condenser and then the diverging beams above the condenser. If the condenser were perfect the rays would focus very accurately at a point above the condenser face, Fig. 16 A. This focal point is where the object should be placed and its distance above the condenser face gives the thickness of the slide to use. One can see that with an optically perfect condenser the thickness should be very exact to get the most brilliant image. If the optical system is less perfect as shown in B Fig. 16 the rays do not all cross at one point, but over an appreciable thickness and anywhere within that elongated focus would give a brilliant illumination. In this case the thickness of the slide used could vary the length of this focus.

In Fig. 16 C the focus is much elongated and the slide might vary greatly in thickness and still give a brilliant image. Above the sectional view of the focus in each case is given a face view of the brightest point as described above in getting the focus of the condenser. One can readily see that the more perfect the focus at a point the smaller will be the point of light, and as all the rays are at that point it will be dazzlingly brilliant, while with B and C, where only part of the rays focus at any given level the circle of light will be less brilliant, but correspondingly greater in diameter. The larger circle of light has the advantage of giving a larger illuminated field, but the disadvantage of loss of brilliancy for the most exacting work. It should be mentioned also that as the focus gives an image of the source of light, the size of the source of light will also affect the size of the bright spot seen in looking down on the image. This is finely brought out by using the sun as a source and the arc light or the incandescent light.

One can see also from these figures that if the slide is too thin the objects will be partly in the dark space between the *converging* beams, and if the slide is too thick a part of the objects will be in the dark space between the *diverging* beams. If one sees the face view with a low power in either case there will be a ring of light and a central dark disc. and will look something like the central stop in Fig. 5 D

As the preparations (blood, saliva, etc.) usually studied by the dark-field method have an appreciable thickness it is better to use a slide somewhat thinner than the optimum where the object is almost exactly at the level of the upper surface. If the slide is somewhat thinner the various levels of the preparation can be focused on by the condenser by slightly raising and lowering it as the case demands. For example, if the optimum thickness is 1 mm. it is better to use slides of 0.90 or 0.95 mm. and if the optimum thickness is 1.55 mm. it is better to use one of 1.50 mm. for ordinary preparations.

Thickness of cover-glass and tube-length. These should be strictl in accordance with the construction of the objective. In all mode objectives the makers state the tube-length and thickness of cover glass for which unadjustable objectives are corrected. As the dark-field illumination brings out very sharply any defects of correction in the objective, one should select a cover of the thickness, and the length of tube recommended by the maker of the objective. This applies particularly to dry, inadjustable objectives. If the objectives are dry and adjustable then corrections can be made for variations from the standard of cover thickness or tube-length.

If the objective being used is homogeneous immersion, the tube-length must be carefully attended to, but the thickness of the cover-glass is immaterial so long as it is thin enough to fall within the working distance of the objective; of course if it were thicker than that one would not be able to get the objective in focus (Bausch, '90; Gage, '87, 1912).

Practical Application of Dark-Field Microscopy

In the practical application of dark-field microscopy it is self-evident that it can be used successfully only with objects scattered, leaving a certain amount of blank or empty space between the objects. If the object being studied covered the whole field then it would all appear self-luminous and give a continuous bright appearance filling the whole field of the microscope.

In Biology, used in the comprehensive sense applied to it by Huxley, there come naturally the following groups of objects in which it is applicable, and likely to yield much information:—

(A) Unicellular organisms in both the plant and the animal kingdoms. This of course would include the Protozoan Animals, the Bacteria, and other unicellular plants.

(B) In the multicellular animals and plants it includes the natural fluid parts with their cellular and granular contents. In the vertebrates, including man, this would, for example, comprise the blood, and the lymph, with their cellular and granular contents; the tissue fluids, and the fluids in the natural cavities like the pericardial, the pleural and the peritoneal cavities, and the liquid found in the cavities of the central nervous system, the joint cavities and tendon sheaths. It is also of great service in the study of the liquids found in mucous containers, as milk, urine, bile, the saliva, the mucous in the nose, and other organs lined with mucous membrane.

Furthermore it is of help in the study of isolated elements of the body like ciliated cells, etc. In a word it is applicable to the study of all animal and vegetable structures—including the pathologic ones—that are naturally isolated, or that can be artificially separated so that there is sufficient blank space between the structural parts.

Dr. Chamot points out its help in the biological examination of water, in the study of foods, fibers, crystallization phenomena, submicroscopic particles and colloids. He adds further (p. 40): "This method is invaluable for demonstrating the presence of very minute bodies or those whose index of refraction is so very nearly the same as that of the medium in which they occur as to cause them to escape detection when illuminated by transmitted light," i.e., by bright-field microscopy.

Summary of Steps Necessary for Successful Dark-Field Observation

1. A powerful source of light must be available.

2. The dark-field condenser is put in place in the substage, and raised until the top is flush with the upper surface of the stage. The condenser is then accurately centered. If there is an iris diaphragm below the condenser it should be made wide open.

3. A homogeneous immersion objective with reducing diaphragm of about 0.80 N.A. is screwed into one of the openings of the nosepiece of the microscope.

4. Slides and cover-glasses of the proper thickness are made very clean, and put in position for rapid handling.

5. The preparation to be examined—blood, saliva, etc.—is mounted on the slide and covered; the cover-glass is sealed with mineral or castor oil, or with shellac cement.

6. The mounted preparation is held in the hand and one or more drops of homogeneous liquid put on the lower side of the slide opposite the cover-glass. The slide is then put upon the stage so that the homogeneous liquid makes immersion contact with the top of the condenser. The condenser may need to be raised or lowered slightly to make the contact perfect.

7. A drop of homogeneous liquid is put on the cover-glass.

8. The mirror is turned until there is a brilliant point of light in the homogeneous liquid on the cover. The objective is then lowered until it dips into the immersion liquid.

9. The microscope is then focused and the light made as brilliant as desired by turning the mirror.

10. Dark-field microscopy requires more accuracy of manipulation than does ordinary microscopy, but the increased visibility pays for all the trouble. A dimly lighted room is desirable for then the eyes are adjusted for twilight vision and can more easily make out the finest details.

Method of Procedure. As an example of the method to be followed in dark-field work, blood may be used. As pointed out nearly 50 years ago, by Dr. Edmunds, blood with dark-field illumination seems like a new structure, so many things are seen with the greatest distinctness that are wholly invisible or only glimpsed when seen by the bright-field method.

(1) Slides of the correct thickness for the condenser are selected and carefully cleaned.

Cover-glasses are also cleaned and placed where they can be easily grasped.

(2) For obtaining the fresh blood the part to be punctured should be cleaned well with 95% alcohol and then with a sterilized needle or Dr. Morre's Haemospast, the puncture is made. The drop of blood exuding can be quickly touched by a cover-glass, and the cover put on the center of one of the prepared slides. If a small amount adheres to the cover, it will spread out in a very thin layer when placed on the slide. At least one preparation should be made which

appears quite red. In making the preparations one should work rapidly so that the various corpuscles will be in their normal numbers, and the fibrin will be formed only after the preparation is on the slide.

If all the preparations are quite red, after a few minutes, one can be made thinner by pressing firmly on the cover by the ball of the thumb covered with gauze or lens paper. The gauze or paper absorbs the blood which runs out at the edge of the cover. In order to prevent evaporation and to help anchor the cover-glass so that it will not move by the pull of the viscid homogeneous immersion fluid, it is advisable to seal the cover by painting a ring of liquid vaseline (petroleum oil) or castor oil around the edge of the cover. One of the thick preparations should not be sealed, but kept for irrigation with normal salt to show especially the fibrin net-work. When ready to study the blood, put a large drop, or two large drops, of homogeneous liquid on the underside of the slide directly opposite the specimen, and place the slide on the stage of the microscope so that the immersion liquid will come over the face of the condenser. Then a drop of immersion liquid is put on the cover-glass and the objective run down into it. If the lighting is secured as explained above one soon learns to focus on the specimen. In general, the field all looks bright just before the objective gets down to the level for seeing the specimen.

(a) The erythrocytes will appear like dark discs with bright rims owing to the convex borders.

(b) The leucocytes appear as real white corpuscles owing to the granules within them which turn the light into the microscope. If the room is moderately warm—20 C or more—the leucocytes, some of them, will undergo the amoeboid movement, and the picture they present will be a revelation to those who never saw it or only with the bright-field microscope. From the clearness with which everything can be seen the minutest change can be followed, and also the most delicate pseudopod detected. Another striking feature will be noticed in the moving ones, that is, the vigorous Brownian movement of the granules in the part of the leucocyte with the amoeboid movement. In those showing no amoeboid movement there is usually no sign of the Brownian movement of the granules; also if a part of the leucocyte is not undergoing amoeboid movement the particles in it are usually motionless.

(c) The fibrin net-work will be seen like a delicate cob-web between the corpuscles. In different parts of the specimen one can find all the appearances of the fibrin shown in text-books on the blood.

(d) Chylomicrons appear everywhere like bright points in the empty spaces. They are in very active Brownian movement. These chylomicrons will probably be the most unusual part to those studying blood with the dark-field for the first time.*

A very striking view of the fibrin net-work may be obtained by irrigating the thick blood preparation. If a drop of normal salt solution is placed on one edge of the cover-glass and a piece of blotting paper on the other the liquid is drawn through washing out many of the erythrocytes. If the washing out process is watched under the microscope the erythrocytes will be seen gliding over or through the fibrin net-work, or some of them will be anchored at one end and if the current is rapid the corpuscles will be pulled out into pear-shaped forms.

The leucocytes look like big white boulders in the stream, wholly unmoved by the rushing torrent around them.

History

Almost always in human progress two steps must be taken (1) The discovery of the fundamental principles involved, and (2) the development of knowledge in other fields to make the application of the principles possible. Often a long time, sometimes a very long time, intervenes between the first steps and the final rendering of the knowledge a part of the common knowledge of mankind. The development of Dark-Field Microscopy is a good illustration of both the statements made.

*The term *chylomicron* is from two Greek words; χῦλός, juice or chyle, μικρόν, any small thing, technically the one-thousandth of a millimeter (μ). I have introduced this word to show the origin of these bodies from the chyle, and to indicate their general average size. Gulliver in 1840–1842, called these minute granules the *molecular base of the chyle* and showed that they were identical in the thoracic duct and in the blood vessels of the same animal. He gave their average size as 1/36,000 to 1/24,000 of an inch. They have been called by others free granules or granulations, elementary particles, etc. In 1896 H. F. Mueller described them as "A never-before observed constituent of the blood" and gave the name of haemoconia, literally, blood-dust. (See Gulliver, Lond. Edin. Phil. Mag. Jan. Feb. 1840; Appendix to Gerber's Anatomy, 1842, and notes in the Works of Hewson, 1846; Mueller, Centralblatt f. allg. Path. u. path. Anatomie, Bd. 7, 1896, pp. 529–539).

The ancient opticians, thousands of years ago, knew well that the principle of contrast was of the highest importance in rendering objects visible; but before this could be applied in microscopy, the microscope itself must be devised. This we see in its simplest form in the convex lenses of Roger Bacon (1266-1267) and in the now rarely used compound form of the Dutch spectacle makers, Jansen and Laprey (1590), composed of a convex objective and a concave ocular (Fig. 17). As a result of the Dutch Compound Microscope, Kepler was led to devise the modern form composed of a convex objective and a convex ocular (1610). But this Keplerian compound microscope has undergone many changes since its first conception and many modifications to render it suitable for giving ability to show the delicate structures in nature with their true appearance. Among these changes may be mentioned the preparation of achromatic lens combinations (Dolland 1757) for telescopes and applied to microscopic objectives between 1820-1830, put on the road to perfection by the introduction of the immersion principle (Hooke 1678, Brewster 1813, Amici 1840-1855) and by the aperture made available by the homogeneous immersion objectives of Tolles 1871-1874, and by the apochromatic objectives of Abbe. Condensers for lighting the object have also played a prominent part from that of Descarts (1637) to those recommended by Brewster (1831) and the homogeneous immersion condensers of Wenham, Tolles (1856 to 1871) and those now regularly made for homogeneous contact with the slide supporting the specimen.

Among the subsidiary discoveries were necessary the arc-light of Davy (1800) and the right-angled arc lamp of Albert T. Thompson (1894) (Fig. 13) and the electric generators now everywhere available. In these last days also the gas filled or Mazda lamps with their close filaments of Tungsten which approximate in brilliancy and compactness of source to the arc lamp and greatly excel it in convenience; and lastly of the production of a glass filter to give the light of the tungsten incandescent lamps true daylight quality, and make microscopic work by this artificial light as comfortable as the light from the northern sky (see Ives 1914, Gage 1915-1916).

The time also between the first appreciation of the dark-field for the study of microscopic objects by Lister (1830), Reade (1838), Wenham (1850), Edmunds (1877), and the appreciation of the microscopical worker in general, came only after the invention of the ultra-

microscope (1903) and the application of the dark-field method to the study and detection of pathologic micro-organisms especially the Spirochaeta pallida (1905). It now promises to give much help in working out the activities and minute details of microscopic structure in animals and plants from the lowest to the highest.

In the earliest stages of microscopic study the objects were seen by the light which they directed toward the microscope, and if over a dark background they appeared with varying degrees of brightness as if self-luminous; but even as early as 1637 (Fig. 17) Descartes microscope had provision for sending the light through the object. In this case much of the light did not reach the object at all, but passed on directly to the microscope. This mode of lighting showed the object more or less as a dark body on a brilliant background.

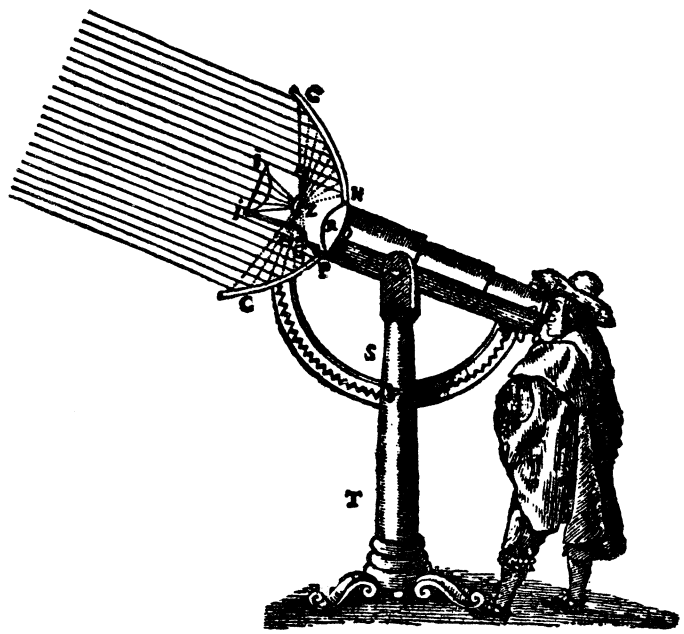

Fig. 17. Descartes Dutch compound microscope with a parabolic reflector and a condensing lens (From Descartes Dioptrique, 1637).

Ocular and Objective. The ocular is a plano-concave lens or amplifier, and the objective (N O P R) is a double convex lens.

Reflector and Condenser. For objects to be lighted from above, there is a parabolic mirror (c c); for those to be lighted from below there is a condensing lens (i i).

These two forms of lighting differed fundamentally in that with the first no light from the source passed into the microscope; but only that from the object, while with the second the light from the source as well as from the object got into the microscope.

The significance of this fundamental difference for the aperture of the objective and for dark-field microscopy were first appreciated by Lister (1830), Wenham (1854), and Gordon (1906), and was practically applied in the manufacture of dark-field apparatus by Zeiss (1904) and Leitz (1905). In a word, it was the appreciation, as stated by Lister (1830) that if the direct light from the source after it had reached the object, were prevented from entering the objective, by blacking the central part of the objective, then only the marginal part of the objective would be functional and that would receive only those rays from the object that were directed to it by the object itself, that is scattered light reflected, refracted, or diffracted, from the object, none of the light from the source getting directly into the microscope. As stated by Wright (p. 217) this is the method of dark-field microscopy by lighting the object with a solid cone of small aperture and, imaging it by hollow beams of large aperture. In practice this method has been discarded for the one by which the object is lighted by beams of light in such a direction with reference to the axis of the objective that none of them can enter the objective directly, and the light going to the microscope comes only from the objects themselves; they will therefore appear self-luminous on a dark background.

The two conditions are (a) where the light is directed upon the object from above and, therefore away from the objective, and (b) where the light is directed upon the object from below, and therefore toward the objective (Fig. 3-4).

If the light is directed upon the object from above and the object is over a non-reflecting background, the object will appear bright in a dark field. Of course, if it is on a light background that will also reflect light into the microscope and both object and background will appear light. It is assumed here that the object or objects cover only a part of the field, leaving plenty of empty space for background.

In striving after a truly non-reflecting background three distinguished men found the same thing, viz., that the only really black thing in nature is a black hole, that is, a space with black walls into which the light cannot enter directly. The dark walls absorb any

stray light, and the empty space gives no reflection. The first of these men devised for his microscopic purposes such a non-reflecting background by means of a small cup or well with the walls painted black. It is known as Lister's black well (1826). The second discoverer was Chevreul (1839), who found in his work on Contrasts that a black space gave the only non-reflecting background. Such a background was used by Marey for making moving pictures to show animal movements. Marey called it Chevreul's black. The third was J. H. Comstock (1901) who found in the study and photography of spider webs that no pigment or fabric was black enough for a background He therefore devised a deep box with the inner walls covered with black velvet and placed it so that the light could not shine into it. Over the mouth of this box the web was placed and lighted at right angles to the opening of the box. The feeble light the webs reflected served well for photography.

These three men then absolutely independently found the same solution to their problem and doubtless many others have found also that Lister's, Chevreul's, and Comstock's black space is the only really black thing in nature.

From the time of Descartes (1637) the means for lighting objects from above the stage have been many. Some of them, like the bull's eye condenser (Fig. 4, lens) and the side reflector send the light only from one side, while with the circular mirror of Descartes (Fig. 17) and the somewhat similar Lieberkuhn reflector (1740) the light is reflected from all sides upon the object. If now the object is on a dark background, it will appear as if self-luminous.

From 1850 to the present two additional means have been devised for lighting from above. The first, following the suggestion of Riddell (1852) aims to make the objective its own condenser, the light being introduced into the side of the objective and reflected down by a small mirror or a prism (H. L. Smith 1865, Tolles 1866). (For a full discussion see W. A. Rogers, Journal of the Royal Micr. Soc., 1880, p. 754-758.)

The other method referred to is that of Prof. Alexander Silverman of the University of Pittsburgh. It consists of a circular electric lamp and reflector which surrounds the objective and shines down upon the object.

Of course all objects lighted from above the stage will give true dark-field effects only when there is a black background, and the objects are scattered, leaving empty space between them.

Dark-Field Microscopy with Substage Illumination. The first specific discussion of the possibility of dark-field microscopy with light from beneath the stage is found in a paper by the Rev. J. B. Reade of Cambridge University and is dated at Peckham, Nov. 1836, and is published as appendix No. 2 in the Micrographia of Goring and Pritchard, 1837. Reade says: p. 229: "To illustrate the two methods (Bright-field and dark-field) by reference to the telescope it may be observed that the discomfort of viewing spots on the sun not unaptly corresponds with the view of microscopic objects on an illuminated field; while the removal of all inconvenient and ineffective light from the field of the microscope corresponds with the clear and quiet view of stars on the dark blue vault of the firmament." He brings out very clearly in his paper that no light from the source shall pass directly into the microscope, only that from the object, and that the object appears "sparkling with exquisite lustre on a jet-black ground."

The first appearance of this method in the general literature of microscopy which was found occurs in John Quekett's Practical Treatise on the Use of the Microscope, 1st ed. 1848, pp. 178-179. He also furnishes a diagram to illustrate the method of lighting something like fig. 4 of the present article, and remarks: "The method consists in illuminating the object by a very powerful light, placed at such an angle with the axis of the microscope that none of the rays can enter it except those which fall directly upon the object, and are so far bent as to pass through it into the compound body," i.e., into the tube of the microscope.

It is referred to in the first edition of W. B. Carpenter's "The Microscope and its Revelations" (1856) as follows:

"Whenever the rays are directed (from below the stage) with such obliquity as not to be received into the object-glass at all, but are sufficiently retained by the object to render it (so to speak) self-luminous, we have what is known as the *black ground illumination;* to which the attention of microscopists generally was first drawn by the Rev. J. B. Reade in the year 1838 (1836-1837) although it had been practised sometime before not only by the author (Dr. Carpenter) but by several other observers."

In addition to the condensing lens of Reade for throwing the very oblique beam of light upon the object, the mirror was used for low powers, and for higher powers, prisms were used especially by Nachet and Shadboldt (1850). It was seen however, that light from only one side might give rise to false appearances.

In the third volume of the Transactions of the Microscopical Society of London, there appeared an epoch-making paper for dark-field microscopy. It is entitled "On the Illumination of Transparent Microscopic Objects on a New Principle." It was read by its author, F. H. Wenham, April 17, 1850. After discussing the prisms of Nachet and pointing out the defect of oblique light from one side only giving rise to false images, he proceeds to show how the defect may be obviated by using two prisms giving light from opposite sides, or, and this is the epoch making part of the paper for dark-field work, by using a truncated parabolic reflector to give a circle of light. A dark stop was present to cut out all but the rays which exceed the aperture of the objective "So that the light which enters the microscope shall be that which radiates only from the object, as if it were self-luminous." The parabolic speculum was truncated so that the light would focus on an object mounted upon the ordinary glass slide.

From this fundamental beginning, illumination by a hollow cone of light by the aid of the truncated parabola, all the advances in dark-ground illumination have proceeded. In 1851, Mr. Shadboldt says: "In order to obviate the objectional shadow (of lighting from one side only) as well as to procure a more brilliant illumination the parabolic condenser was projected by Mr. Wenham, to whom alone belongs the credit of having suggested the use of oblique illumination in *every azimuth*, so as to produce a black field." In this paper Mr. Shadboldt commends the use of a condenser made wholly of glass and depending upon internal reflections to take the place of the metallic parabolic mirror of Wenham. This he named a spheroannular condenser. In considering the obliquity required to have all of the light going to the object of an angle to fall outside the aperture of the objective, it seems to Shadboldt highly desirable that each objective to be used in dark-field work should have its own special condenser. That he understood as perfectly as we the possibility of using a single condenser for all objectives is shown by the following quotation, p. 157, "It is highly desirable that the

condenser should be constructed specially with reference to the aperture of the object-glass with which it is intended to operate; and for a reason to be given immediately, it will be seen that cutting off some of the rays, in order to make a condenser work with objectives of very much larger aperture, although quite practicable and even generally in use with the parabolic condenser, is not nearly so advantageous as the use of a separate condenser for every object-glass . . . of high power at least."

In 1856 Mr. Wenham himself advocates the use of a truncated paraboloid of solid glass with a central stop to cut out all the central rays which would not be internally reflected from the upper surface of the paraboloid. He brings out in the clearest manner possible the need of using immersion contact with the paraboloid to permit the very oblique rays to pass out of the paraboloid into the overlying substance. If the object is in water, then water immersion and when the object is mounted in balsam, he advocates the use of an immersion liquid between the glass slide and the paraboloid of camphine, turpentine or oil of cloves as their refractive index is nearly the same as crown glass and permits the passage of the rays of great aperture to pass on into the slide and the balsam containing the objects. We now use cedar oil or other homogeneous liquid for the same purpose.

In 1877 Dr. James Edmunds presented before the Quekett Microscopical Club a paper on "A New Immersion Paraboloid Illuminator." It consisted of a paraboloid of glass cut off at an exactly calculated distance below the focus, this distance varying in the four lenses which constituted his set, and the plane top being made optically continuous, and as nearly as possible optically homogeneous with the substance of the slide, by means of a cementing fluid of high refractive index, such as anhydrous glycerine, castor oil, copaiba-balsam, oil of cloves, etc. The paraboloid lenses acted on the principle of total internal reflection, and each one was calculated for the thickness of the slide beneath which it was to be used (1/16th in 1/100 inch slides) so as to converge upon the object all of the light entering the base of the paraboloid. Parallel light should be thrown into the base of the paraboloid, and the most splendid effects were obtained by means of direct sunlight. Water immersion objectives of 1/16th and 1/8th inch focus were used. After speaking of some test objects he says, p. 19: "With bacterial fluids, the effect

was equally remarkable. Saliva, blood, etc., viewed by a good dry quarter of about 95° (NA 74), were seen almost as new objects when lighted up by this paraboloid."

As it was recognized from the time of Reade that to gain the dark-field effect the light going to the object must be of an obliquity so great that it could not enter the microscope directly; this involved either a paraboloid or other dark-field illuminator of such great range that it might be used with all objectives, or the suggestion of Shadboldt must be followed that each objective have a paraboloid especially constructed to give it the best possible effect. This question naturally became very insistant when the water immersion objectives of large aperture came into use, and especially when the homogeneous immersion objectives came into common use (1880-1890). It has finally been settled by adopting the first possibility, viz., the use of dark-field illuminators adapted for all objectives, the aperture of the objectives being reduced, where too great, to a point somewhat below 1.00 NA. This makes it possible to utilize a ring of light between 1.00 and 1.52 NA for the dark-field illumination, and this ring of light produced by the sun or the electric light has been found sufficient for practically all dark-field microscopy. It should be stated in passing that the ring of light produced by the dark-field illuminators usually falls between 1.00 NA, and 1.45 NA. Some fall below 1.00 NA and some only go to 1.30 or 1.35. The reducing diaphragms for homogeneous immersion objectives which have come to the writer with objectives have ranged from 0.40 NA to 0.80 NA.

From 1907-1910 papers were written describing and figuring reflecting condensers made on the cardioid principle to take the place of the truncated paraboloid in dark-field work. The effort was made to so figure the component segments of glass that the spherical and chromatic errors would be largely eliminated, and that the entire ring of light could be brought to a more perfect focus than is possible with the truncated paraboloid: that is, to be optically more like A than like B or C in Fig. 16. A simple plate form for use on the top of the stage has also been devised. When this is used the substage condenser is turned out so that the light can pass directly up from the plane mirror to the condenser. This form is not easy to keep accurately centered. From the writer's experience with quite a variety of these dark-field condensers in biological work

the paraboloids have proved the easiest to work with and the most generally satisfactory.

As a final word,—now that the means have been found for fuller microscopic revelations, it behooves biologists to make the most of them; and in the study of the finest details in living things by this dark-field lighting, perhaps a truer conception of structure and action can be gained than by a too exclusive dependence on dead material treated with the endless variety of fixers and stains.

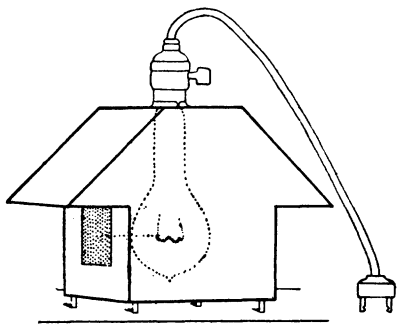

Fig. 18. Chalet microscope lamp for bright-field microscopy (Two-fifteenths natural size).

The lamp has two daylight-glass windows under the overhanging roof. The roof serves to shade the eyes. The source of light is a 100 watt Mazda C lamp bulb, the filament of which is centered with the windows.

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1914. Substage illumination by hollow cones. Jour. Quekett Micr. Club, Vol. XII, (1914) pp. 301-308. 3 pl.

BAUSCH, Edward

1890. The full utilization of the capacity of the microscope and means for obtaining the same. Proc. Amer. Soc. Microscopists, Vol. XII, 1890, pp. 43-49.

Among other matters Mr. Bausch gives a very thoughtful discussion of the effect of the cover-glass and of tube length.

BORELLUS, PETRUS

1655. De vero Telescopii inventore, cum brevi omnium conspiciliorum historia. Ubi de eorum confectione, ac usu, seu de effectibus agitur, novaque quaedam circa ca proponuntur. Accessit etiam centuria observationum microcospicarum. Authore Petro Borello, regis christianissimi conciliario, et medico ordinario. Hagae-Comitum, ex typographia Adriani Vlacq, MDCLV (1655).

Evidence from those with personal knowledge that telescopes and microscopes were made by the Dutch spectacle makers, Zacharias Jansen, and Hans Laprey, 1590.

CARPENTER, WILLIAM B.

1856. The Microscope and its Revelations. First edition 1856.

An admirable statement of dark-field microscopy is given with the apparatus devised up to that time for effecting it. Showing how greatly dark-field microscopy had been discarded in England one can compare the first and the 6th (1856-1881) editions of this work with the 8th edition, (1901).

CHAMOT, EMILE MONNIN

1915. Elementary Chemical Microscopy. New York, 1915.

This work is recommended not only for the account given of dark-field microscopy and its application, but for the ultra-microscope, the polariscope, the micro-spectroscope and indeed all other chemico-physical apparatus used with the microscope, and their application in chemical and physical investigations.

CHEVREUL, M. E.

1838. De la Loi du Contraste simultané des Couleurs et de l'assortiment des objets colorés considéré d'apres cette loi. Paris, 1839. Work written 1835-1838. Third English edition, 1890. Part of Bohn's Scientific Library.

COMSTOCK, J. H.

1912. The Spider Book. A manual for the study of spiders and their near relatives, the scorpions, pseudoscorpions, whip-scorpions, harvestmen, and other members of the class Arachnida, found in America north of Mexico; with analytical keys for their classification and popular accounts of their habits. New York.

In this book are given pictures of the spider webs photographed against a black space, i.e., a deep box lined with black velvet. See p. 181. The first photographs made in this way were taken in 1901. They were exhibited before the Entomological Society of America at its first meeting, Dec. 28, 1906.

CONRADY, A. E.

1912. Resolution with dark-ground illumination. Jour. Quekett Micr. Club, Vol. 11, (1912) pp. 475-480.

He says: "To get the utmost resolving power with dark-ground illumination, the condenser must have not less than three times the NA of the objective. If the condenser has less than three times the aperture of the objective then the limit of resolution is found by taking ¼ the sum of the apertures of objective and condenser: e.g., if cond. has NA of 1.40, and of obj. 1.00 NA, their sum is 2.40, ¼ of 2.40 = 0.60 NA; limit in this case."

COX, HON. JACOB D.

1884. Robert B. Tolles and the angular aperture question. Proc. Amer. Soc. Microscopists, Vol. VI, (1884) pp. 5-39.

This very able address, one of the ablest our society ever had the fortune to hear from its president, brings out with absolute clearness and fairness the steps in progress and the role played by Robert B. Tolles in actually making possible the final step, and taking that step, in his homogeneous immersion objectives. That is not all, he published the formula by which the objectives were made. The reading of this address is most strongly recommended to our younger members.

DESCARTES (LAT. CARTESIUS), RENÉ

1637. Oeuvres, Publiées par C. Adam et P. Tannery sous les auspices du ministère de l'instruction publique, Vols. I-XII. Paris, 1902.

The Dioptrique is in Vol. 6 of this edition, and the French and the figures are as in the original of 1637. In Cousin's edition the figures are often considerably modified and the French modernized.

DOLLOND, JOHN

An account of some experiments concerning the different refrangibility of light. Read June 8, 1758. Philos. Trans. Roy Soc. Lond. 1758, pp. 733-743. This is the original paper on achromatic telescopes, etc.

EDMUNDS, JAMES

1877. On a new immersion paraboloid illuminator. Jour. Quekett Micr. Club, Vol. V, (1877) pp. 17–21. Monthly Micr. Jour., Vol. XVIII, 1877, pp. 78–85.

The Paraboloid was made optically continuous and as nearly as possible, optically homogeneous with the slide by the use of anhydrous glycerin, castor oil, copaiba-balsam or oil of cloves. He says that saliva, blood, and bacterial fluids gave remarkable effects, and were almost like new objects when seen with this paraboloid.

GAGE, S. H.

1917. The Microscope, an introduction to microscopic methods and to histology. 12th revised edition, Ithaca. 1917.

GAGE, S. H. and H. P.

1914. Optic Projection. Principles, installation and use of the magic lantern, the projection microscope, etc. Ithaca, 1914.

GAGE, S. H.

1887. I. Microscopical tube-length and the parts included in it by the various opticians of the world. II. The thickness of cover-glass for which unadjustable objectives are corrected. Proc. Amer. Soc. Microscopists, Vol. IX, 1887, pp. 168-172.

This paper gave the information that has led to greater uniformity.

GAIDUKOV, N.

1910. Dunkelfeldbeleuchtung und Ultramikroskopie in der Biologie und in der Medizin. 5 plates, 81 pages. Jena, 1910.

There is a bibliography of books and papers covering 9 pages (202 titles).

GORDON, J. W.

1907. The top-stop for developing latent power of the microscope. Jour. Roy. Micr. Soc., 1907, pp. 1-13. See also Wright, pp. 216-217.

The plan is to cut out all of the central beam by a stop at the eye point instead of by opaqueing the central part of the objective.

GORING AND PRITCHARD

1837. Micrographia, containing practical essays on reflecting, solar, oxy-hydrogen gas microscopes, micrometers, eye-pieces, etc. 231 p. Many figures in the text, one plate. Whittaker & Co., Ave-Maria-Lane, London, England. 1837. Rev. J. B. Reade on dark-field, pp. 227-231.

HALL, JOHN CHARLES

1856. On an easy method of viewing certain of the Diatomaceae. Quart. Jour. Micr. Sci., Vol. IV, (1856) pp. 205-208.

In this paper Dr. Hall figures natural size, the "spotted lens" of that time, i.e., a very thick, more than hemisphere of glass with the central part opaqued. (See Quekett, 3d. ed., p. 135 where it is said that it is the invention of Thomas Ross.) Hall used this spot lens for oblique light with the ordinary bright field microscopy. He expresses astonishment that this instrument, designed to give dark-field effects, should give bright ones. He did not consider the fact that the aperture of this spot lens was insufficient to throw all the light outside of the aperture of the objective. One would get the same effect if a wide-angled homogeneous immersion were used with a paraboloid, and no reducing diaphragm were put into the objective.

HEIMSTÄDT, OSKAR

1907. Neuerungen an Spiegelkondensoren (Aus der optischen Werkstätte von C. Reichert in Wien). Zeit wiss. Mikr., Bd. XXIV, (1907) pp. 233-242.

HEIMSTÄDT, OSKAR

1908. Spiegelkondensor und Paraboloid. Zeit. wiss. Mikr., Bd. XXV, (1908) pp. 188-195. Erwiderung an Herrn O. Heimstädt, by Siedentopf, pp. 195-199.

Dr. Heimstädt objects to some of Dr. Siedentopf's statements in his paper, "Die Vorgeschichte der Spiegelkondensor." Perhaps the spirit of the polemic will best be brought out by a quotation from Heimstädt, p. 188. "Vol allem beeinträchtigt es den Wert und auch die Neuheit dieser Dunkelfeldbeleuchtung nicht im geringsten, dass dabei längst vergessene Methoden älterer englische Optiker wieder verwendt wurden." In a word, it is well brought out in these papers where the fundamental ideas came from.

IGNATOWSKY, W. V.

1908. Ein neuer Spiegel-kondensor. Zeit. wiss. Mikr., Bd. XXV, (1908) pp. 54-67 with figures of the substage and the plate form. See also Jentzsch, and Siedentopf. Jour. Roy. Micr. Soc., London, 1911, pp. 50-55.

JENTZSCH, DR. FELIX

1911. The reflecting concentric condenser. Physikalische Zeitschrift, Bd. XI, pp. 993-1000. See also Ignatowsky and Siedentopf, Jour. Roy. Micro. Soc., 1911, pp. 50-55.

KEPLER, JOHANNES

1604. Opera Omnia, Vol. II. Ad Vitellionem Paralipomena. (De modo visionis et humorum oculi usu.) 1604, pp. 226-229. 11 figs.

Correct dioptrics of the eye here given, and also the explanation of the effect of convex and concave spectacles.

1611. Dioptrica.—Demonstratio eorum quae visui et visibilibus propter conspicilla non ita pridem inventa accidunt, pp. 519-567. 35 figs., 1611.
The amplifier, real images, and erect images. The Keplerian microscope (Modern microscope.)

LISTER, JOSEPH JACKSON
1830. On some properties in achromatic object-glasses applicable to the improvement of the microscope. Philos. Trans. Royal Society London, Vol. 120 (1830) pp. 187-200.
On p. 191 he discusses the effect of a "Stop behind the object-glass" (retro-objective stop) by which only the outer zone of the objective is used, the central zone being stopped out. See Wenham, 1854.

MAREY, ETIENNE JULES
1901. The history of Chronophotography. Annual Report of the Smithsonian Institution for 1901, pp. 317-340.
On p. 320 Marey refers to Chevreul's method of obtaining perfect blackness.

MAYALL, JOHN, JUN.
1885. Cantor Lectures on the Microscope. Lectures delivered before the Royal Society of Arts, Nov. Dec. 1885.
On pp. 95-96 are given the facts regarding the working out and production of homogeneous immersion objectives. Tolles is given due credit.

MOORE, DR. V. A.
1897. The Hemospast, a new and convenient instrument for drawing blood for microscopic examination. Trans. Amer. Micr. Soc., Vol. XIX (1897) pp. 186-188.
After using this "spring needle lancet" individually and with large classes for many years I quite agree with Dr. Moore when he remarked to me the other day, "It is the most humane instrument I have ever seen for drawing blood." I would like to add to this: And one of the most efficient.

QUEKETT, JOHN
1848. A practical treatise on the use of the microscope including the different methods of preparing and examining animal, vegetable and mineral structures.
First edition, 1848. Reade's method given and illustrated pp. 178-179; Second edition, 1852, Reade's method illustrated pp. 194-195. Third edition, 1855, Reade's method, the method of Wenham, Spot-Lens method of Thomas Ross, the methods of Schadboldt and Nobert are all given.

READE, REV. J. B.
1837. On a new method of illuminating microscopic objects, pp. 227-231 of Goring and Pritchard's Micrographia, which see. (1837).

ROGERS, WM. A.
1880. On Tolles' interior illuminator for opaque objects. (With note by R. B. Tolles). Jour. Roy. Micr. Soc. London., Vol. III (1880) pp. 754-758.
In this paper Rogers gives the history of the devices for making the objective its own condenser by introducing light into its side and reflecting the light down upon the object.

SHADBOLT, GEORGE

1851. Observations upon oblique illumination; with a description of the author's Sphaero-annular condenser. Trans. of the Micr. Soc. of London. Vol. III, pp. 132, 154.

This paper was read in 1851. As this condenser is like the glass paraboloids now used for dark-field work, they are often called the Wenham-Shadbolt paraboloids. Shadboldt discusses prisms in this volume.

SIEDENTOPF, H.

1907. Paraboloid-Kondensor, eine neue Methode für Dunkelfeldbeleuchtung zur Sichtbarmachung und zur Moment-Mikrophotographie lebender Bakterien, etc. Zeit. wiss. Mikr., Bd. XXIV, (1907) pp. 104-108.

1907. Die Vorgeschichte der Spiegelkondensoren. Zeit. wiss. Mikr., Vol. XXIV (1907) pp. 382-395. 16 figures are given of early forms.

1908. Mikroskopische Beobachtungen bein Dunkelfeldbeleuchtung. (Mitteilung aus der optischen Werkstätte von C. Zeiss, Jena) Zeit. wiss. Mikr. Bd. XXV (1908), pp. 273-282. Two plates of photomicrographs of the rays above the different condensers. See also under Heimstädt.

1910. Cardioid-Condenser. Jour. Roy. Micr. Soc. Lond., 1910, pp. 515. See also Ignatowsky, and Jentzsch, Jour. Roy. Micr. Soc. Lond., 1911, pp. 50-55, where will be found a statement concerning the historical relation of these different condensers.

STEPHENSON, J. W.

1879. A catoptric, immersion illuminator. Jour. Roy. Micr. Soc. Lond., Vol. II (1879) pp. 36-37.

This condenser does not depend on internal reflection, but by a silvered surface around the central part. According to Siedentopf this is the condenser copied by Reichert; and according to Heimstädt Wenham's truncated paraboloid was copied by Zeiss (See under Heimstädt).

WENHAM, F. H.

1850. On the illumination of transparent microscopic objects on a new principle. Trans. Micr. Soc. Lond., Vol. III. (1850) pp. 83-90.

This is the paper by Wenham in which dark-field illumination is produced by a hollow silvered parabolic speculum.

1854. On the theory of the illumination of objects under the microscope with relation to the aperture of the object-glass, and properties of light; with practical methods for special differences of texture and colour. Quart. Jour. Micr. Sci. Vol. II (1854) pp. 145-158.

In this paper Wenham refers to the method of Lister (1830) for darkening the central zone of the objective so that no light can enter the outer zone, unless, as Wenham says, it is "*radiated*" from the object (See his fig. 1, and pp. 149-150 of the article). On p. 153, in the reference to the effect that his paper of 1850 had had in the microscopical world he says, "As proof of the utility and correctness of my theory, I have only to mention the many applications of it that have since that time (between 1850 and 1854) come into general use, in the way of adapting central stops to the achromatic condenser, single (i.e., "spot lenses") and compound lenses, etc."

1856. On a method of illuminating opaque objects under the highest powers of the microscope. Trans. Micr. Soc. Lond. in Quart. Jour. of Micr. Sci., Vol. IV, (1856) pp. 55-60.

It is in this paper that Mr. Wenham insists on making homogeneous contact with the slide and the top of the paraboloid. It will be noticed that in this paper he speaks of Opaque Objects, while in the paper of 1850 he speaks of Transparent Objects. By reading the two papers it will be seen that many of the objects mentioned in the two papers are identical. This gains an explanation from the fact that he has apparently given up the notion that the objects were visible by their own "radiated" light, but by the light they reflect to the microscope. Consequently he represents (Fig. 4) the light from the condenser going to the cover-glass and being reflected from it down upon the object and he says that it makes the most perfect kind of a Lieberkuhn reflector. One can see instantly that when homogeneous immersion objectives are used there can be no total reflection from the cover.

WOOD, ROBERT W.

1911. Physical Optics. New and Revised Edition, 1911.

On p. 373, he discusses, "Penetration of the disturbance into the second medium," and shows that going back to the time of Newton and Fresnel, it was known that while there was total reflection, the light seemed to pass for a minute distance into the rarer medium. This explains why one may get a brighter dark-field picture than is expected if objects are in optical contact with the slide.

WRIGHT, SIR A. E.

1907. Principles of Microscopy, being a handbook to the microscope. London and New York, 1907.

The writer has found this book the best and most thought-provoking of any that has been published on the microscope during the last 50 years.